ON THE ULTRASTRUCTURE OF THE STRIATED BORDER OF MIDGUT DIGESTIVE CELLS OF *APIS MELLIFERA* AND *MELIPONA QUADRIFASCIATA ANTHIDIOIDES* (HYMENOPTERA, APIDAE)[1]

Carminda da Cruz-Landim[2]
José Eduardo Serrão[2]
Regina Lúcia M. Silva de Moraes[2]

ABSTRACT

The microvilli of the midgut digestive cells of bees *Apis mellifera* L. and *Melipona quadrifasciata anthidioides* Lepeletier are numerous and exceptionally elongated. They are large at the base, narrowing to the tip and supported by microfilaments that extend into the cortical cytoplasm of the cell, where they are not arranged in the characteristic terminal web. In their tip the cytoskeleton is weakly developed. These features confer an undulated aspect to the microvilli, whose thin sections can be seen as sheets of different electron densities.

KEYWORDS. Bees, microvilli, midgut, ultrastructure.

INTRODUCTION

The midgut epithelium of insects is constituted of some different cell types such as columnar, regenerative, endocrine, globet and oxyntic cells. Bees present the first three cell types, the most abundant of which are columnar cells (CRUZ-LANDIM, 1985; SERRÃO, 1995). Typically, columnar cells have a striated border in their apical surface, consisting of a regular array of cylindrical microvilli. This structure is common in absorptive cells and serves to increase the area by which the digestive food can enter the cell (NOIROT & NOIROT-TIMOTHÉE, 1972; BIGNELL **et al.**, 1982; CRUZ-LANDIM, 1985). In addition to their absorptive

1. Supported by CNPq and FINEP.
2. Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista, Campus de Rio Claro, Caixa Postal 199; CEP 13506-900, Rio Claro, SP, Brasil. Fax 0195 34 0009.

function, columnar cells are also related to digestive enzymes secretion (CHAPMAN, 1975). Usually, the microvilli are arranged in a hexagonal pattern and contain a core of microfilaments extending to the cytoplasm of the cell. Moreover, they are covered with a fuzzy material referred to as glycocalix (KITAJIMA, 1975). Interesting variations in this basic pattern have been reported in some insect orders (O'LOUGHLIN & CHAMBERS, 1972; BILLINGSLEY & DOWNE, 1983).

In this study, we report the ultrastructure of the striated border of midgut digestive cells of *Melipona quadrifasciata anthidioides* and *Apis mellifera*.

MATERIAL AND METHODS

The specimens analyzed were adult workers of *Melipona quadrifasciata anthidioides* Lepeletier and *Apis mellifera* Linnaeus. Voucher specimens were deposited in the bee collection of the Departamento de Zoologia, Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, Brazil.

Digestive tracts from workers were removed into buffered saline solution for insects, and their midgut isolated. The pieces were fixed in 2.5% glutaraldehyde in 0.1M Na cacodylate buffer at pH 7.2, washed twice in the buffer, post-fixed in 1% osmium tetroxide in the same buffer, dehydrated in a series of increasing concentrations of ethyl alcohol, and embedded in Epon-Araldite resin, following usual procedures. Ultrathin sections obtained with glass knives were stained with uranyl acetate and lead citrate, and examined and measured (μm) in an electron microscope.

For the calculation of the approximate increase of the apical surface area achieved by the presence of the microvilli, the following formula was used: $\mathbf{S = L \cdot 2\pi \cdot D/2 \cdot N}$ (NOIROT & N.-TIMOTHÉE, 1972) in which: **S** = augmentation of the surface area; **L** = microvilli length; **D** = microvilli diameter; **N** = number of microvilli per μm^2.

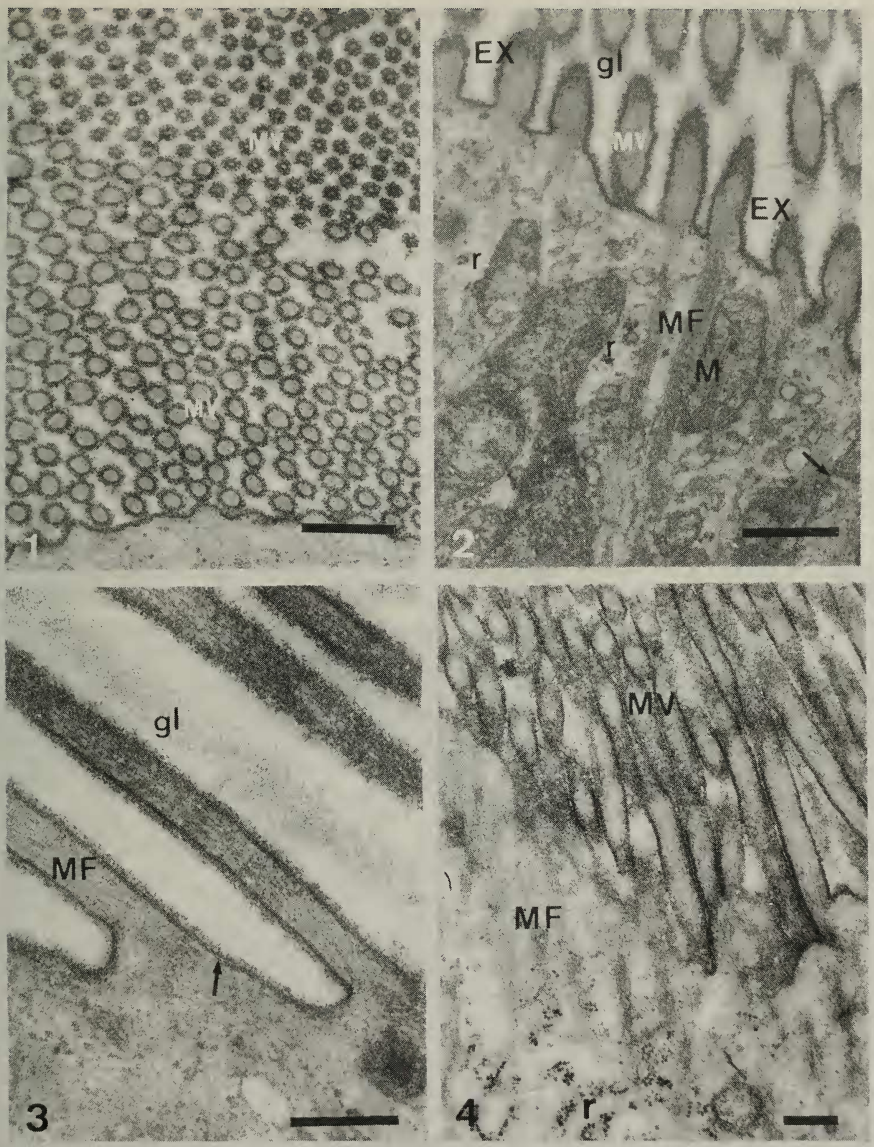
The isolated midgut was fixed in Karnovsky solution (2.5% glutaraldehyde + 2.0 % paraformaldehyde), sectioned in pieces belonging to the anterior, median and posterior regions, and macerated in 1% osmium tetroxide for 48 h. The pieces were dehydrated, passed through the critical point desiccation, covered with gold and examined in a Jeol P15 scanning electron microscope.

RESULTS AND DISCUSSION

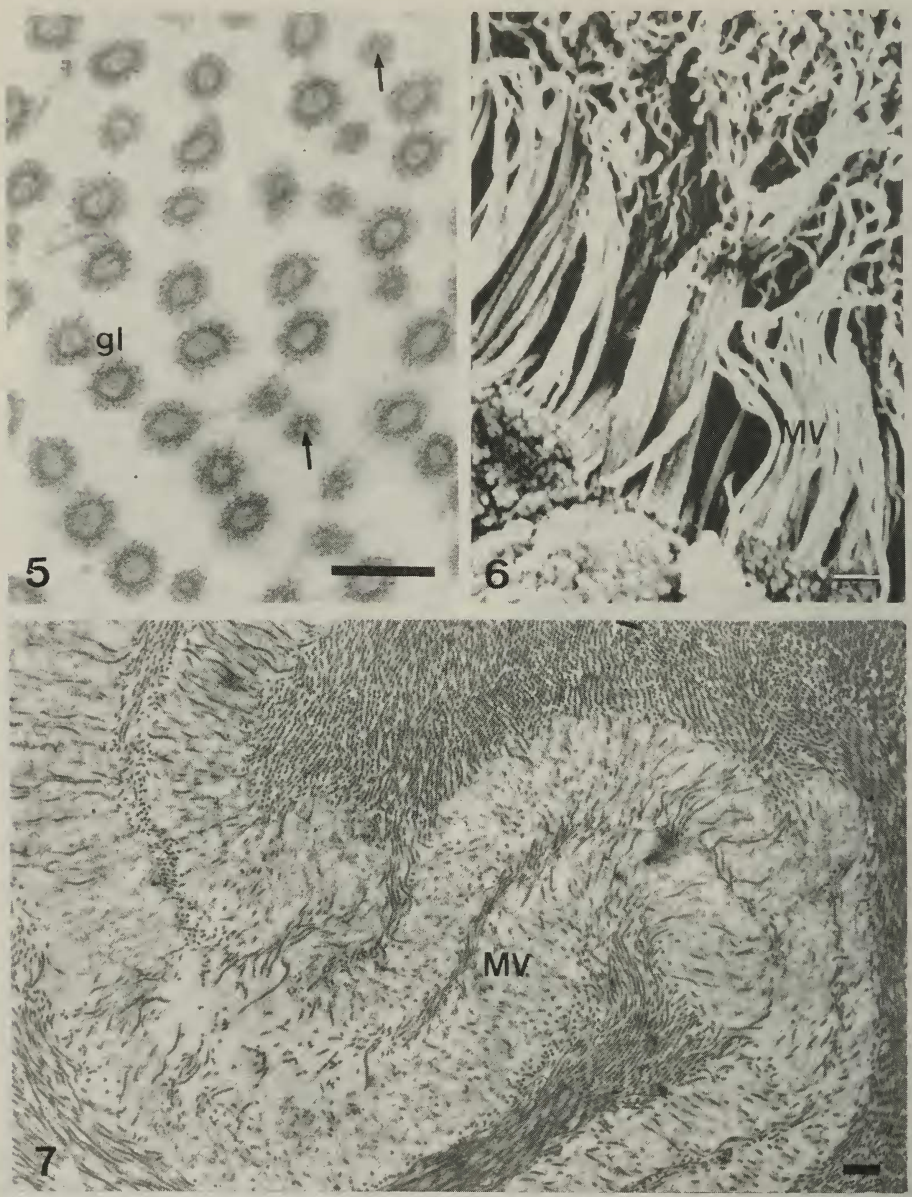
No significant differences were found between the two species studied so reported observations apply to both species.

The microvilli of midgut columnar cells of bees were exceptionally elongated structures (17-22 μm in length). They were thicker in the base (0.12 μm in diameter) than in the tip (0.03 μm in diameter) (fig. 1). Their number per μm^2 is 30-40. With the formula of NOIROT & N.-TIMOTHÉE (1972), it can be seen that the augmentation of the surface area achieved by microvilli in the bees is 135-220X.

A comparison with microvilli measurements for the cockroaches *Periplaneta americana* Linnaeus and *Blaberus craniifer* Burmeister, the termites *Cephalotermes rectangularis* Sjötedt, *Microcerotermes edentatus* Wasmann, *Cubitermes severus* Silvestri and *Kalotermes flavicolis* Fabricius and the beetle *Tenebrio molitor* Linnaeus (NOIROT & N.-TIMOTHÉE, 1972; BIGNELL **et al.** (1982) show that the increase of the surface area in adult bees is more than 3 times as much as in those insects. This greater increase can be related to nutrient absorption, since bees do not posses gastric caeca, a midgut specialization to nutrient absorption. The microvilli of bees were not so closely packed and therefore did not display hexagonal arrangement. In their origin the adjacent microvilli were separated by



Figs. 1-4. Microvilli of digestive cell of *Melipona quadrifasciata anthidioides*: 1, microvilli in transversal section (MV), note the diameter variation along their length; 2, apical surface of the digestive cell showing microvilli (MV) separated by extracellular space (EX) with glycocalix (gl) weakly developed. Microtubules (arrow), mitochondria (M), microfilaments (MF), ribosomes (r); 3, microvilli in longitudinal section showing the support of microfilaments (MF) and the glycocalix (gl). Plasmatic membrane (arrow); 4, apical region of the digestive cell showing the microfilaments (MF) in the cortical cytoplasm and the absence of the terminal web. Microvilli (MV), ribosomes (r). Bars 0.5 µm., figs. 1,2,4; 0.2 µm, fig. 3.



Figs. 5-7. Microvilli of digestive cell of *Apis mellifera*: 5, microvilli in transversal section, notice that in the tip (arrows) the cytoskeleton is weakly developed, glycocalix (gl); 6, scanning electronic micrograph showing the undulated aspect of the ending part of the microvilli (MV); 7, aspect of the bent microvilli (MV) whose thin section can be seen as sheets of different electron-densities. Bars 0.5 μm, figs. 5,7; 1 μm, fig. 6.

an extracellular space of 0.2-0.8 μm, which where the glycocalix was weakly developed (fig. 2). In this region, they were supported by microfilaments which extended for up to 2 μm into the cellular cortical cytoplasm, where they were not arranged in the characteristic terminal web (figs. 3, 4). In the tip the microvilli, the cytoskeleton was weakly developed (fig. 5). These features confer an undulated aspect to the ending part of the microvilli (fig. 6).

The relatively great extension of the core of microfilaments supporting the microvilli into the apical cytoplasm may be an adaptation to the length of the microvilli. In addition, the typical terminal web reported in vertebrates (BRUNSER & LUFT, 1970) was never detected in insects (NOIROT & N.-TIMOTHÉE, 1972, BIGNELL **et al.**, 1982).

The microvilli of bee midgut presents an intermediary morphology between characteristic microvilli and stereocilia. A distinguishing feature of microvilli is their enlarged basal portion, supported by microfilaments and glycocalix, while the characteristics of stereocilia are their length, the narrowed tip and the lack of stiffness in this region. Because of their length, their small diameter in the tip and the absence of microfilaments, the microvilli bend to form undulations whose thin sections can be observed as sheets of different electron-densities (fig. 7). Under lower resolution, this aspect can give the false impression that a delamination of the apices of epithelial cells has occurred. These sheets were described as type I of peritrophic membrane origin in bees (SNODGRASS, 1956, VECCHI & BRAGAGLIA, 1965, DAVIDSON, 1970, MELLO **et al.**, 1971, CRUZ-LANDIM & MELLO, 1981). However, ultrastructural data have pointed out that the peritrophic membrane is always seen as a single layer and its origin is suggested to occur at the anterior end of the midgut or cardia (SERRÃO, 1995).

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